Review

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Epithelial–mesenchymal transition in chronic liver disease: Fibrogenesis or escape from death?

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The possibility that epithelial–mesenchymal transition (EMT) could contribute to hepatic fibrogenesis in chronic liver diseases as reported in other organs, particularly the kidney, reinforced the concept that activated hepatic stellate cells were not the only key players in the hepatic fibrogenic process and that other cell types, either hepatic (i.e. portal fibroblast) or extrahepatic (bone marrow-derived cells and circulating fibrocytes) could contribute to this process. The possibility of the rapid mobilization of a large amount of fibrogenic cells by EMT after liver tissue injury made this phenomenon a relevant and suitable target for anti-fibrogenic strategies. Following an initial enthusiasm for the discovery of this novel pathway in fibrogenesis and the publication of a several highly quoted papers, more recent research has started to cast serious doubts upon the real relevance of this phenomenon in human fibrogenetic disorders. The debate on the authenticity of EMT or at least on its real contribution to the fibrogenic process has become very animated, sometimes reaching levels of "religious" integralism. The overall result is a general confusion on the meaning and on the definition of several key aspects. The aim of this article is to analyze and discuss the evidence supporting or confuting this possibility in order to reach reasonable and useful conclusions.

Introduction

Epithelial–mesenchymal transition or transformation (EMT) is defined as a key developmental program characterized by loss of cell adhesion, repression of E-cadherin expression, and increased cell mobility [1]. The term "transition" is currently preferred since it reflects in part the reversibility of the process. Indeed, the phenotypic plasticity afforded by an EMT is characterized by the occurrence of the reverse process, i.e. a mesenchymal–epithelial transition (MET), which involves the conversion of mesenchymal cells to epithelial derivatives. EMT is essential for numerous developmental processes including mesoderm formation and neural tube formation. Its potential clinical relevance was initially highlighted and characterized in cancer development and metastasis and, more recently, in chronic inflammatory/fibrogenic disorders.

EMTs: different types for different purposes?

Upon EMT, epithelial cells assume a more mesenchymal phenotype, characterized by increased cell motility. This possibility does not seem to be restricted to epithelial cells during development or to cancer cells but has been shown to occur in wound healing, tissue regeneration, and organ fibrosis. A classification has been recently proposed to distinguish between these different types of EMT [2]: (a) type 1 EMT, associated with implantation, embryo formation, and organ development, is characterized by a program organized to generate diverse cell types that share common mesenchymal phenotypes. In turn, type 1 EMT can generate the primary mesenchyme that has the potential to subsequently undergo a MET to generate secondary epithelia; (b) in type 2 EMT, the program begins as part of a repair-associated event that has been suggested to generate fibroblasts and other related cells in order to reconstruct tissues following trauma and inflammatory injury. Differently from the other types of EMT, type 2 EMT is associated with tissue damage and inflammation and, accordingly, it is abrogated once these causative events cease; (c) type 3 EMT occurs in neoplastic cells that have previously undergone genetic and epigenetic changes, specifically in genes that favor clonal outgrowth and the development of localized tumors. In this context, EMT is elicited by several oncogenic pathways (Src, Ras, integrin, Wnt/beta-catenin and Notch) [3]. In particular, Ras-MAPK has been shown to activate two related transcription factors known as Snail and Slug [4]. Both of these proteins are transcriptional repressors of E-cadherin and their expression induces EMT. Activation of the phosphatidylinositol 3' kinase (PI3K)/AKT axis is also emerging as a central feature of EMT [5]. Other relevant pathways in EMT observed in the metastatic process include the transcription factor Twist and FOXC2, an important player during embryonic development [6,7], as well as the involvement of microRNAs [8].

The possibility that EMT, specifically type 2 EMT, could contribute to hepatic fibrogenesis in chronic liver diseases was suggested by studies that closely followed experimental evidence obtained in other organs and particularly the kidney [9–11]. This biological option strengthened the concept that activated hepatic

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stellate cells (HSC) were not the only key players in the hepatic fibrogenic process and that other cell types, either hepatic (i.e. portal fibroblast) or extrahepatic (bone marrow-derived cells and circulating fibrocytes) could contribute to this process [12–14]. The possibility of the rapid mobilization of a large amount of fibrogenic cells by EMT after liver tissue injury made this phenomenon a relevant and suitable target for anti-fibrogenic strategies. Following an initial enthusiasm for the discovery of this novel pathway in fibrogenesis and the publication of a several highly quoted papers, more recent research has started to cast serious doubts about the real relevance of this phenomenon in human fibrogenetic disorders.

The aim of this article is to analyze and discuss the evidence supporting or confuting this possibility in order to reach reasonable and useful conclusions.

EMT in fibrogenetic disorders: background issues and "specific" cell markers

Before addressing specific experimental evidence supporting or not the occurrence of EMT in hepatic fibrogenesis, there are two major pieces of background information that need to be highlighted. The first concerns the almost automatic and uncritical parallels drawn between the morphology and behavior of locomotory or sedentary cells *in vitro* and in different pathological processes *in vivo*. *In vitro* data supporting EMT derive from cells in a highly artificial two-dimensional milieu in which there is no vascular, endocrine, or neurologic contribution. On the other hand, studies performed in three-dimensional matrices also do not recapitulate the real tissue complexity and the results are very much affected by the applied experimental conditions. An additional technical problem concerns the fact that most of the *in vitro* studies are performed with epithelial cells that in most circumstances belong to transformed cell lines cultured in an abundance of serum factors conditioning the loss of their epithelial features and the progressive acquisition of "mesenchymal" characteristics.

Within this background, a multitude of studies have shown that epithelial cells, including hepatocytes, when cultured *in vitro* retain epithelial features including polarity and specific protein expression (i.e. albumin for hepatocytes), but when chronically stimulated with TGF-β1 or serum factors acquire a pattern of gene expression that is somehow typical of myofibroblasts *in vivo* and in the mesenchyme during development [15–19]. These genes are often represented by *Slug*, *Twist*, *Snail*, *α-SMA*, *vimentin*, *desmin*, fibroblast-specific protein 1 (*FSP1*; also known as S100A4 and MTS-1), and discoidin domain receptor tyrosine kinase 2 (*DDR2*).

Some of these markers have been used to identify epithelial cells that are in the midst of undergoing an EMT associated with chronic inflammation. Such cells continue to exhibit epithelial-specific morphology and molecular markers, such as cytokeratin and E-cadherin, but often show the concomitant expression of the fibroblast specific protein-1 (FSP-1) and α-SMA. These aspects have been proposed to represent the intermediate stages of EMT, when epithelial markers continue to be expressed but new mesenchymal markers have already been acquired, and, overall, these observations have led to the notion of the so-called "partial EMT" [2].

A second key issue is the value of different cell markers in defining cell-lineage and, even more relevant, cell function. The most widely used marker identifying myofibroblasts is the cytoskeletal protein α-SMA that is part of the contractile machinery and is involved in cell motility. In adult normal tissue α-SMA expression is mostly restricted to vascular smooth muscle cells, but in most chronic inflammatory and fibrogenic disease states is often found in myofibroblasts of different derivation, and this expression is interpreted as an active involvement of these cells in fibrogenesis (i.e. "activated myofibroblast"). Accordingly, α-SMA cannot be a good lineage marker since its expression is activated by disease states and, in addition, does not denote function since α-SMA expression has little or no role in the synthesis and deposition of fibrillar extracellular matrix (ECM). Regardless of this, there is supportive evidence that epithelial cells express intermediate filaments such as α-SMA and vimentin following tissue injury [20,21]. As it will be further expanded, this is likely due to the engagement of epithelial cells into an adaptive response to injury that includes the activation of several new programs of gene expression leading to the acquisition of a migratory phenotype. An additional popular cell marker is the multifunctional calcium binding protein FSP-1 (member of the S100 family) wrongly defined as "fibroblast-specific". Indeed, this protein, originally described in malignant epithelial cells as a facilitator of metastatization by regulating the function of contractile proteins [22,23], is not "fibroblast-specific" and is expressed predominantly by activated macrophages [24–27]. Therefore, although this protein is expressed in cultured epithelial cells exposed to TGF-β, it cannot be considered as a marker of myofibroblast or epithelial lineage. Finally, the use of other proposed markers such as Snail, Twist, Slug seems to be of limited value since they are readily expressed by epithelial cells, myofibroblasts, and macrophages following tissue injury and, although they may be relevant for cell motility and activation, do not denote cell lineage.

Overall, in a context of tissue fibrogenesis where type 2 EMT is supposed to occur, the most appropriate marker of myofibroblast function *in vivo* is the synthesis and deposition of pathological type I collagens [24,28]. However, the evaluation of this property is not as easy as it could superficially appear and is mainly hampered by the fact that collagens are predominantly extracellular proteins. Several *in vitro* studies have shown that different types of epithelial cells, including hepatocytes, activate the *Collagen1α1* gene in culture. Similar findings have been obtained in leukocytes and endothelial cells [29,30]. However, all these cell types do not generate *Collagen1α1* transcripts *in vivo* [24,28]. An additional interpretative problem is related to the fact that some cell types and particularly macrophages are able to internalize ECM with consequent intracellular accumulation of collagen which is not a sign of collagen synthesis. For example, the combination of FSP-1 and intracellular collagen positivity in activated macrophages detected in tissue sections may lead to the wrong identification of epithelial cells undergoing EMT and contributing to fibrogenesis [29].

To summarize these considerations, the expression of several often wrongly defined "myofibroblast-specific" markers is rather irrelevant since the only pathophysiologically relevant feature of fibrogenic cells, irrespective of their origin, is the synthesis and deposition of relevant amounts of fibrillar collagen.

Developmental rationale for fibrogenic EMT in different tissues: is there a rationale for the liver?

As already mentioned before, the epic of EMT in the fibrogenic process involving the liver as well as of other organs and tissues was launched by initial evidence obtained in the kidney [9–11]. In this

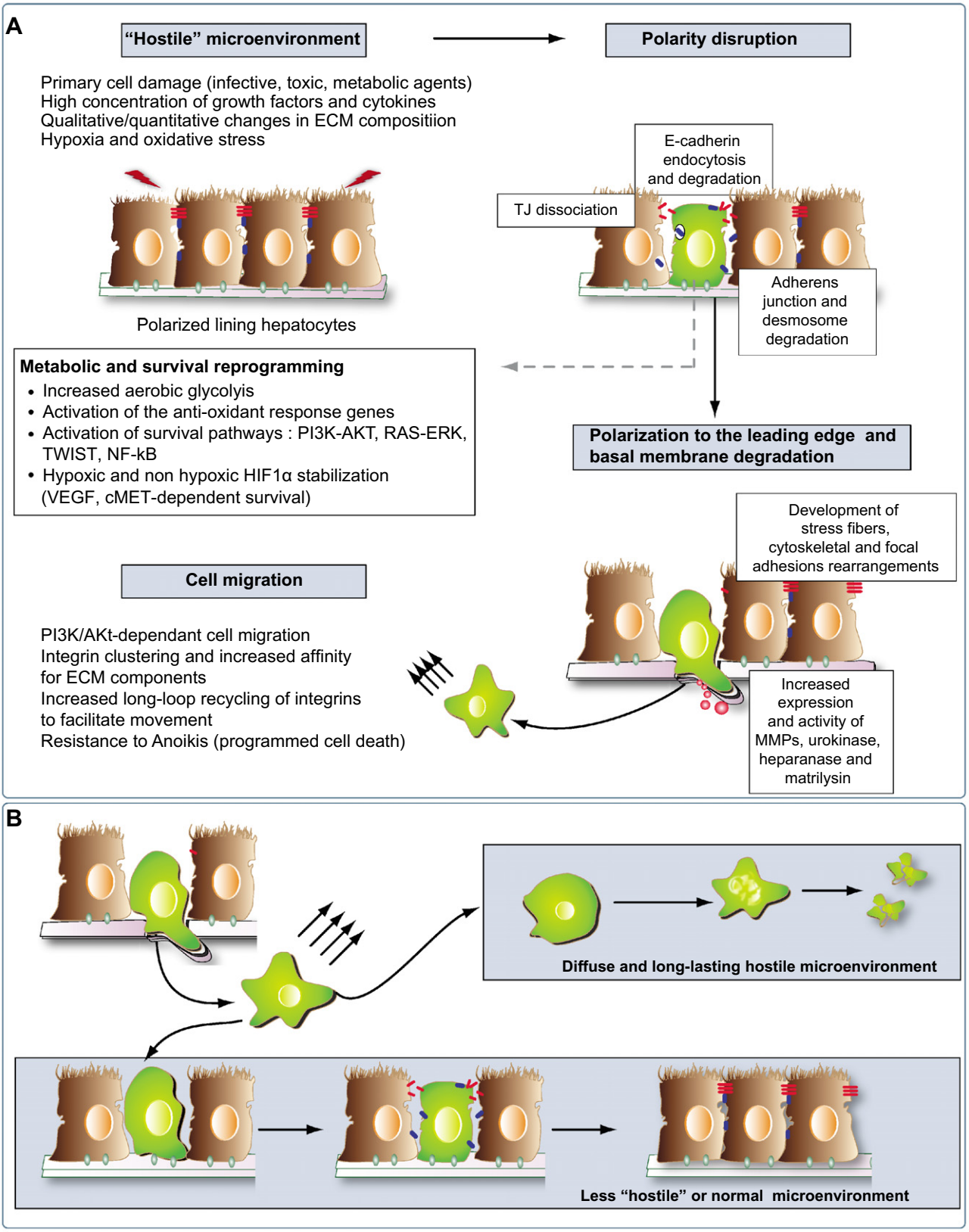
connection, the rationale for type 2 EMT derives from the development paradigm of mesoderm-derived mesenchymal cells developing in epithelial cells according to the process called MET. The entire epithelium of the kidney, including the tubular epithelial cells, is derived from the intermediate mesoderm during the development of the urogenital system via a MET. This represents a peculiar aspect of renal biology: by retaining some imprint of their mesenchymal origins, kidney epithelial cells may be particularly prone to return to this state, via the EMT that occurs in response to inflammatory stress and leads to pathologic fibrosis. While this developmental course is clearly recognized in the kidney, it is not involved in the development of gastrointestinal organs such as the liver and the pancreas, where all the epithelial cells have been shown to derive from the foregut endoderm [31,32] and, in this context, the mesoderm contribution to the development of the liver and pancreas seems to be limited to stromal cells including hepatic and pancreatic stellate cells, respectively [33,34]. Therefore, a developmental rationale for EMT as a source of myofibroblasts in liver and pancreas is at best very limited.

The saga of EMT investigation in animal models of liver fibrosis: what have we learned?

The inadequacy of cell markers for the identification of unequivocal EMT in fibrogenic disorders and the criticisms raised against evidences obtained *in vitro* prompted major efforts to characterize this potentially relevant phenomenon in animal models of liver fibrosis. The methodological approach used to overcome the limitation of cell surface or cytosolic cell markers is the so-called "lineage tracing" or "fate mapping", which is based on the concept that once a heritable marker is activated *in vivo*, it is permanent and not inducible. In other words, the marker will be present in any cell deriving from the labeled cell regardless of differentiation, proliferation, and migration in the context of tissue injury. However, the application of this concept is only achievable with a complex methodology potentially bearing pitfalls and even more complex interpretative issues. For example, the activation of the heritable marker should be a feature only of the cell type to be labeled and should not normally occur in disease. In this context, the most likely candidates to activate a heritable marker are transcription factors that are active developmentally.

Zeisberg and co-workers were the first to report *in vivo* evidence for hepatocyte EMT [35]. These authors employed a methodology to drive the DNA recombinase Cre under the control of a 2.3 Kb fragment of the albumin promoter/enhancer locus in order to activate the heritable marker *LacZ* in *Rosa26R* mice developing CCl_4-induced liver fibrosis, so that all hepatocytes and potentially derived cells were irreversibly tagged with β-galactosidase. In this model, recombination was specific for hepatocytes but was, however, incomplete. Regardless of this, the main interpretative issue in this work was related to the sole use of FSP-1 marker to detect scar forming myofibroblastic cells: although it was evident that a detectable percentage of *LacZ* protein β-gal-positive cells co-expressed FSP-1, there was no conclusive evidence that these cells were effectively myofibroblasts (absence of α-SMA positivity, no evidence of collagen production). This last fundamental issue was subsequently addressed by Taura and co-workers [28], who employed a similar *Alb-Cre* transgenic model in the *Rosa26R, LacZ* expressing reporter mouse with the additional use of the *Coll-GFP* reporter to simultaneously identify collagen-producing cells and hepatocyte-derived cells in the injured liver. The results of this study suggested that FSP-1 positive cells do not generate collagen *in vivo* and that collagen producing cells are α-SMA and PDGF-receptor β-positive. These observations, although not related to human chronic liver diseases in terms of both genetic background and injury model strongly argue against the contribution of hepatocyte EMT to liver tissue fibrosis.

It is a common observation that bile duct basement membranes undergo degradation in fibrogenic liver diseases and that cholangiocytes, the other major hepatic epithelial cell type, assume fibroblast-like, non-cuboidal shapes. Therefore, with the new wave of EMT and liver fibrosis, it became obvious that the next step was to investigate whether or not biliary cells could undergo EMT in chronic liver disease. It is well established that proliferating cholangiocytes within the so-called "ductular reaction" (i.e. "reactive cholangiocytes"), detectable in all types of chronic liver disease, express a variety of pro-fibrogenic growth factors and cytokines (for example PDGF-B chain, TGF-β1, ET 1, MCP-1) and are likely to contribute to fibrosis and inflammation by promoting activation, proliferation, and collagen synthesis in the surrounding pro-fibrogenic cells [36–42]. Nevertheless, the possibility of a direct contribution of cholangiocytes to fibrosis via EMT was suggested by the report by Omenetti and co-workers showing *in vitro* a complete EMT in an immature cholangiocyte cell line treated with activated HSC conditioned medium [43]. Concomitantly, other authors reported the co-expression of epithelial and mesenchymal markers in cholangiocytes in human liver obtained from patients with different types of cholestatic disease [44,45]. The authenticity of cholangiocyte EMT was recently challenged with the methodology previously used for the investigation of hepatocyte EMT. Along these lines, Scholten and co-workers employed the *Cre-Lox* technology for lineage tracing and studied several mouse strains expressing *Cre* under cholangiocyte-, HSC-, or FSP-1-specific promoters in two established models of liver fibrosis, i.e. chronic CCl_4 intoxication and common bile duct ligation (BDL) [46]. In this case the fundamental experiment was tracing the fate of cells expressing K19, a bile ductular cell-specific marker, after permanent genetic *Cre*-mediated labeling of cholangiocytes. The key result of this study was that, although myofibroblast markers were often found in the close proximity of the K19 + progeny of cholangiocytes, the two signals never overlapped in either CCl_4 or BDL fibrosis. Based on these and other observations reported in the paper, the authors concluded that cholangiocyte EMT does not occur in their experimental models. Additional data reported in the study by Scholten and co-workers provided parallel evidence that MET, i.e. the transition from myofibroblast to cholangiocyte in the specific experimental context, is also not likely to occur. This latter conclusion is equally of high value since, at some point of this saga, some authors have proposed that, in an ideal perspective of "liver cell panplasticity", HSC could be transitional cells derived from epithelial cells that have undergone partial EMT [47] or even a particular type of oval cell/hepatocyte precursor [48]. This hypothesis was based, at least in part, on the finding of an adult subpopulation of primary rat HSC expressing the progenitor cell marker CD133 and differentiating into either myofibroblasts or hepatocytes when cultured under different *in vitro* conditions [49]. This latter observation, besides its implications in the issue of MET, raises additional questions on the homogeneity of the HSC population; moreover, the results of the study by Cassiman and co-workers [50] should also be considered as they


A
"Hostile" microenvironment
Polarity disruption
Primary cell damage (infective, toxic, metabolic agents)
High concentration of growth factors and cytokines
Qualitative/quantitative changes in ECM compositiion
Hypoxia and oxidative stress
Polarized lining hepatocytes
E-cadherin endocytosis and degradation
TJ dissociation
Adherens junction and desmosome degradation
Metabolic and survival reprogramming
• Increased aerobic glycolyis
• Activation of the anti-oxidant response genes
• Activation of survival pathways : PI3K-AKT, RAS-ERK, TWIST, NF-kB
• Hypoxic and non hypoxic HIF1α stabilization (VEGF, cMET-dependent survival)
Polarization to the leading edge and basal membrane degradation
Development of stress fibers, cytoskeletal and focal adhesions rearrangements
Cell migration
PI3K/AKt-dependant cell migration
Integrin clustering and increased affinity for ECM components
Increased long-loop recycling of integrins to facilitate movement
Resistance to Anoikis (programmed cell death)
Increased expression and activity of MMPs, urokinase, heparanase and matrilysin
B
Diffuse and long-lasting hostile microenvironment
Less "hostile" or normal microenvironment

clearly showed that HSC do not descend from the neural crest but may derive from multiple sources such as the septum transversum mesenchyme, the endoderm or the mesothelial liver capsule.

It should be reminded that the methodologies employed in the "lineage tracing" studies bear potential pitfalls. As already highlighted in a recent commentary [51], the Cre-Lox system is a very elegant tool to demonstrate that lineage conversion does occur but it does not prove conclusively that this does not happen. Going back to the study by Scholten and co-workers [46], since only 40% efficacy of Cre-recombination was achieved, it is possible that only a specific subset of cholangiocytes/ductular cells was traced and, therefore, it is not possible to exclude that EMT may have occurred in the remaining population involved in the ductular reaction characterized by a weak or absent K19 expression and potentially including liver progenitor cells. In order to resolve this important issue, Chu and co-workers undertook lineage tracing studies using Alfp-Cre × Rosa26-yellow fluorescent protein (YFP) mice, a model enabling to track the behavior of virtually all bipotential epithelial progenitors and their progeny in liver injury [52]. These authors investigated the co-localization of YFP with the markers such as S100A4, vimentin, α-SMA, or pro-collagen 1α2 in the BDL (2,4, and 8 wks), CCl_4 (3 wks), and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC; 2 and 3 wks) models of liver fibrosis. In no case, they found evidence of co-localization although the above "mesenchymal" markers were abundantly present in the peribiliary regions. Therefore, these data add additional evidence against the possible transformation of cholangiocytes and hepatocytes into fibrogenic cells. In any case, it is important to note that the evaluation timeframe in any mouse model is very short when compared to the fibrogenic process occurring in human liver diseases and that mouse models do not display reactive bile ducts with features identical to those present in human disease.

To conclude this paragraph, it is relevant to delineate the current advancement in injury activated EMT in the kidney, the organ where EMT was first described and where there is indeed the greatest developmental rationale for this process. Using two distinct epithelial cell-specific drivers, two distinct reporters and two different models of renal fibrosis, Humphreys and co-workers found no evidence that epithelial cells become myofibroblasts *in vivo* [25]. In addition, this study highlighted the possibility that in the kidney all myofibroblasts derive from a discrete population of PDGFRβ+/CD73+ perivascular pericytes that are derived from the metanephric mesenchyme and show similarities with stellate cells of the liver and pancreas [53].

The concept of EMT in organ fibrosis and cancer: need for a more flexible interpretation

The complexity of EMT and its recognition as a relevant biological phenomenon in organ fibrosis and cancer requires a more flexible and somehow compromising interpretation in order to fit into a logical scheme. Indeed, even in cancer development and metastasis, the occurrence of EMT is currently extensively challenged [54]. In this context it is argued that disorderly differentiation, loss of cell polarity, loss of lineage specific or tissue specific cytologic features, and acquisition of a migratory/invasive phenotype are typical aspects of carcinoma. In other words, what is called EMT is simply the result of a genetic reprograming of cancer cells aimed at ensuring one of the fundamental aspects of cancer which is cell motility and invasion. The acquisition of cell morphology and the expression of cytoskeletal and surface proteins involved in cell motility typical of mesenchymal cells does not transform a cancer cell into a myofibroblast. Accordingly, EMT in cancer biology is not necessarily seen as a phenomenon where cancer cells differentiate into myofibroblasts to create a tumor-associated fibrotic stroma. This latter is mostly created by the recruitment and activation of local myofibroblasts and circulating fibrocytes in response to soluble factors released by cancer cells and by the disruption of the normal ECM caused by membrane-anchored or secreted metalloproteases in the process of cancer invasion [55,56].

In the injury-activated EMT described in chronic fibrogenic disorders, epithelial cells are likely to undergo a similar genetic reprograming which is not caused by genetic mutations typical of cancer but rather due to an adaptive response to injury and to the changes occurring in the milieu of a chronically damaged tissue. As a consequence, epithelial cells acquire features allowing the acquisition of cell motility. When the occurrence of these "transition" features is evaluated with the expression of cell markers classically but unreliably attributed to myofibroblasts, the most likely result is to reach the even more unreliable conclusion that epithelial cells have "cosmetically" transformed into pro-fibrogenic cells. In this framework, the lack of a convincing demonstration of active collagen synthesis remains a critical point supporting the unreliability of this conclusion. Now, a spontaneous question arises: why non-transformed epithelial cells in a context of tissue injury should become motile and "escape" from the original epithelial lining? A possible hypothesis could derive from the so-called "redox-based escape mechanism from death" which has been shown to govern crucial steps of the metastatic process [57]. This adaptive response is now recognized to occur also in non-transformed epithelial cells in a context of inflammation, hypoxia, and oxidative stress. Overall, this process can be viewed as an integrated "escape program" from a hostile microenvironment mainly triggered by redox changes but also by the presence of an altered ECM composition and high concentrations of growth factors and cytokines involved in the chronic wound healing reaction. The program is characterized by changes in cell metabolism and cell motility aimed at promoting pro-survival choices and escape from oxidative damage (Fig. 1A). The acquisition of the motile phenotype implies adequate

Fig. 1. Hypothesis of the "redox-based escape mechanism from death" to explain the acquisition of a migratory phenotype of hepatocytes in a context of tissue injury. (A) Normal polarized lining hepatocytes are exposed to different environmental factors creating a hostile microenvironment. In some hepatocytes, this leads to an epigenetic re-programing leading to polarity disruption, loss of intercellular and ECM adhesions, activation of aerobic glycolysis, and survival pathways. The acquisition of the motile phenotype implies an adequate cytoskeletal reorganization with the expression of proteins such as α-SMA and vimentin, the polarization of cytoskeletal structures to the leading edge of movement and the degradation of basal membrane like-structures through an increased expression and activity of membrane-anchored or secreted metalloproteases, urokinase, heparanase, and matrilysin. Once the cell has "escaped" from the lining, motility is ensured mostly by PI-3K/AKT signaling, integrin clustering, and recycling associated with increased affinity for the surrounding ECM components, as well as the activation of a degradative potential toward these components. In this context, integrin activation through ECM-cell contacts, in addition to being indispensable for cell movement, is also a key factor ensuring resistance to *anoikis*, a form of programmed cell death which is induced by anchorage-dependent cells detaching from the surrounding ECM. (B) The possible final outcomes of the escape process are basically two: the motile epithelial cell reaches a less hostile microenvironment and undergoes a reprograming toward the original lining phenotype or, in the presence of a diffuse and long-lasting hostile milieu, the newly acquired "escaping" features progressively run out and the cell loses the resistance to *anoikis* with the consequent induction of apoptosis.

Key Points

- Epithelial-mesenchymal transition or transformation (EMT) is defined as a key developmental program characterized by loss of cell adhesion, repression of E-cadherin expression, and increased cell mobility. The concept of EMT involves phenotypic plasticity and imply the occurrence of the reverse process, i.e. a mesenchymal-epithelial transition (MET).
- Three types of EMT have been proposed: type 1, typical of development, type 2, occurring in chronic fibrogenic disorders, and type 3, characteristic of cancer and metastasis.
- The occurrence of EMT relies mostly on the expression of cell markers often believed to be exclusive of myofibroblasts. However, in a context of tissue fibrogenesis where type 2 EMT is supposed to occur, the most appropriate marker of myofibroblast function *in vivo* is the synthesis and deposition of pathological type I collagens.
- Several *in vitro* studies have shown that different types of epithelial cells, including hepatocytes, activate the *Collagen1a1* gene in culture. Similar findings have been obtained in leukocytes and endothelial cells. However, all these cell types do not generate *Collagen1a1* transcripts *in vivo*.
- The methodological approach used to overcome the limitation of cell surface or cytosolic cell markers is the so-called "lineage tracing" or "fate mapping", which is based on the concept that once a heritable marker is activated *in vivo*, it is permanent and not inducible.
- In their complex, studies employing this methodological approach have documented, in different animal models of liver fibrogenesis, that some hepatocytes or cholangiocytes acquire "mesenchymal markers" implicated in cell motility and survival, but are not involved in active fibrillar ECM deposition and, therefore, cannot be considered pro-fibrogenic cells.
- Overall, the most evident feature of EMT is the acquisition of a motile phenotype by epithelial cell in injured tissue. Hypothetically this could be viewed as an integrated "escape program" from a hostile microenvironment mainly triggered by redox changes but also by the presence of an altered ECM composition and high concentrations of growth factors and cytokines involved in the chronic wound healing reaction. The program is characterized by changes in cell metabolism and cell motility aimed at promoting pro-survival choices and escape from oxidative damage.
- The escape program has two possible two outcomes: the motile epithelial cell reaches a less hostile microenvironment and undergoes a reprogramming towards the original lining phenotype or, in the presence of a diffuse and long-lasting hostile milieu, the newly acquired "escaping" features progressively run out and the cell undergoes apoptosis.
- In both cases there is a clear pathophysiological advantage compared to the fate of non escaping cells, whose most likely fate is to undergo cell necrosis with further release of reactive oxygen species and other pro-fibrogenic and pro-inflammatory molecules.
- Accordingly, what has been defined type 2 EMT, could be a process aimed at limiting fibrogenesis rather than being a pro-fibrogenic event.

cytoskeletal reorganization with the expression of proteins such as α-SMA and vimentin, the polarization of cytoskeletal structures to the leading edge of movement, integrin clustering and recycling associated with increased affinity for the surrounding ECM components, and the activation of a degradative potential toward these components. This latter feature involves an increased expression and activity of membrane-anchored or secreted metalloproteases, urokinase, heparanase, and matrilysin. In this context, integrin activation through ECM-cell contacts, in addition to being indispensable for cell movement, is also a key factor ensuring resistance to *anoikis*, a form of programmed cell death which is induced by anchorage-dependent cells detaching from the surrounding ECM. The possible final outcomes of the escape process are basically two (Fig. 1B): (a) the motile epithelial cell reaches a less hostile microenvironment and undergoes a reprograming toward the original lining phenotype (a possibility that, according to the above mentioned "cosmetic" interpretation is viewed as MET), or (b) in the presence of a diffuse and long-lasting hostile milieu, the newly acquired "escaping" features progressively run out and the cell loses the resistance to *anoikis* with the consequent induction of apoptosis. In both cases there is a clear pathophysiological advantage compared to the fate of non-escaping cells, whose most likely fate is to undergo cell necrosis with further release of reactive oxygen species and other pro-fibrogenic and pro-inflammatory molecules [58]. Accordingly, what has been defined type 2 EMT could be a process aimed at limiting fibrogenesis rather than being a pro-fibrogenic event.

In conclusion, the knowledge relative to the discovery, characterization, and interpretation of what is defined as EMT in chronic fibrogenic disorders of the liver represents a scientific treasure that has prompted discussion, animated debates and has ultimately provided further maturity in this field of research. Definitely, there is now need for a more insightful analysis of the real pathophysiological meaning of these observations beyond their superficial or "cosmetic" features.

Conflict of interest

The author declared that he does not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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